REMARKS/ARGUMENTS

Claims 1, 2, 6, and 7 are pending. Claim 1 has been amended, but no new matter has been introduced by way of the amendments to the claim.

Applicants acknowledge and thank Examiner Samala for the indication on pages 2 and 3 of the Office Action that the rejection of claims 1-7 under 35 U.S.C. § 112, second paragraph and the rejection of claims 1, 2, 4, and 7 under 35 U.S.C. § 102(b) were both withdrawn. The only remaining rejection in the instant application is the rejection of claims 1, 2, 6, and 7 under 35 U.S.C. § 103(a) over Mattrey (WO00/45855) in view of Dugstad *et al.* (U.S. Patent No. 6,221,337). Applicants submit the remarks that follow and the accompanying declaration by Dr. Per C. Sontum ("the Sontum declaration") in support of the patentability of the rejected claims.

I. *Interview Summary*

The undersigned and the Applicants wish to thank Examiners Samala and Wu for the cordial and productive interview of March 8, 2011. The Examiners' helpful comments and suggestions were instrumental in preparing this response. During the interview, Applicant's representatives discussed the rejections under 35 U.S.C. § 103(a) and claim amendments that may overcome the art rejection. Specifically, the undersigned discussed possibly presenting evidence that the microbubble compositions used in the claimed method are significantly more stable than the microbubble compositions described in Mattrey. The Examiners agreed to reconsider the 103(a) rejections in light of such evidence.

II. *The Rejection Under 35 U.S.C. § 103(a) Should Be Withdrawn*

Claims 1, 2, 6, and 7 stand rejected under 35 U.S.C. § 103(a) over Mattrey (WO00/45855) in view of Dugstad *et al.* (U.S. Patent No. 6,221,337) for the reasons set forth on pages 3-7 of the Office Action. Applicants respectfully traverse this rejection for the reasons that follow.

Claim 1 is directed to a method for the identification of a sentinel lymph node in a subject. The method comprises administering to the subject a composition comprising microbubbles. The microbubbles comprise a shell and a gas or gas precursor. The microbubbles have a mean particle size of about 0.25-15 μm in diameter and a pressure

stability of at least 50% at a pressure of 120 mm Hg. Notably, the shell comprises negatively charged phospholipids in an amount of from 50-100%. The claimed method further comprises allowing the microbubbles to accumulate in the sentinel lymph node. The microbubbles are subsequently detected in the sentinel lymph node using ultrasound.

As will be discussed in greater detail below, and in the Sontum declaration, Mattrey does not disclose, suggest or otherwise contemplate using phospholipid-containing compositions bearing an overall negative charge. Nor does Mattrey appreciate the unexpected stability of such compositions when they are exposed to pressure before, during, and after administration. It would therefore not have been obvious to specifically pick microbubbles comprising at least 50% negatively charged phospholipids to specifically identify sentinel lymph nodes, as presently claimed; even in view of Dugstad, who admittedly discloses microbubble dispersions comprising phospholipids having an overall positive[1] or negative net charge, albeit not for specifically indentifying sentinel lymph nodes.

The Patent Office should consider that Mattrey provides a **large** range of options for contrast agent microbubbles purportedly useful for identifying the sentinel lymph node, including microbubbles made out of proteins, cyanoacrylate, palmitic acid, surfactants, and neutrally or near-neutrally charged phospholipids, such as MRX115 and Imagent®.[2] *See* Mattrey at page 16. As the Patent Office will appreciate, the shell of the MRX115 microbubble comprises phospholipids and polyethyleneglycol-phospholipids bearing an overall **neutral charge** and the shell of Imagent® comprises phospholipids bearing an overall **neutral charge**. Applicants respectfully submit that the only way that one of skill in the art would pick microbubbles comprising at least 50% negatively charged phospholipids to specifically identify sentinel lymph nodes is by using impermissible hindsight.

In studies that Dr. Sontum personally conducted in 1995, he found that microbubble compositions comprising neutrally or near-neutrally charged phospholipids, such as MRX115 and Imagent®, where not nearly as stable, under pressure, as microbubble compositions comprising overall negatively charged phospolipids, such as Sonazoid®.

[1] In paragraph 15 of his declaration, Dr. Sontum admits that electrostatic stabilization may also be achieved using positively charged lipids, as Dugstad suggests. However, as blood proteins are predominately negatively charged, such positively charged microbubbles would display excessive protein opsonization after administration. This opsonization would lead to activation of the body's immune system and would cause a pronounced adverse, and perhaps toxic effect *in vivo*.

[2] Mattrey's example, in fact, focuses on Imagent® (AFO150).

Sonazoid®, as the Patent Office will appreciate, is at least one embodiment that falls within the scope of claim 1.

The data from Dr. Sontum's study (see paragraphs 10-12 of the attached Sontum declaration), in fact, unexpectedly showed that Sonazoid® retained more than 95% of its initial attenuation efficacy after pressurization at a pressure of up to 300 mm Hg and was thus significantly and unexpectedly more pressure stable than products like MRX 115 and Imagent®, both of which are disclosed in Mattrey.

As Dr. Sontum discusses in paragraphs 11 and 12 of the attached declaration, pressure stability is a predictor of (i) the functionality of the various products for identification of sentinel lymph nodes; and (ii) microbubble destruction during imaging. During subcutaneous injection hydrostatic pressures typically reach more than 300 mm Hg. Under those pressures, significant amounts of the microbubbles in MRX115 and **all** of the microbubbles in Imagent® are destroyed during injection.[3] Accordingly, significant amounts of such microbubbles are destroyed **prior to** imaging. Exposure to ultrasound is equivalent with exposure to applying pressure to the microbubbles. Ultrasound exposure will destroy even more microbubbles made from phospholipids and having an overall neutral or near-neutral charge, such as MRX115 and Imagent®. *See*, *e.g.*, Moran *et al. Ultrasound in Med. & Biol. 26*: 629-639 (2000) (attached hereto as Exhibit A). In contrast, microbubbles comprising at least 50% negatively charged phospholipids, such as Sonazoid®, not only will survive injection, but will also survive the imaging procedure.

According to Dr. Sontum, the unexpectedly high stability of microbubbles comprising at least 50% negatively charged phospholipids, such as Sonazoid®, is probably due to the negative charge of the shell lipids. While not being bound by theory, the negative head groups leads to electrostatic repulsive intermolecular forces within the stabilizing shell. Upon stress-induced deformation (*e.g.*, as during pressure stress from injection, *in vivo* pressures; and ultrasound exposure) these charges will be brought out of their thermodynamic equilibrium state resulting in a restoring force that (i) will limit further deformation and (ii) will restore the original equilibrium once the stress is relieved. A high density of negatively

[3] One should wonder, therefore, if Mattrey was actually able to image the popliteal lymph nodes using Imagent® as he purports to do on page 35, lines 25-28.

charged shell components will therefore inhibit deformation due to stress and increase the stability of the microbubble.

In contrast, shells made from predominately neutral lipids, such as with MRX115 and Imagent®, little or no electrostatic repulsion will exist between the head groups. Such shells will deform more easily and, as shown in the data included in the Sontum declaration, will also disintegrate more easily. This evaluation is corroborated by the fact that Imagent®, which contains 100% neutral lipids, displays the lowest stability of the three agents that Dr. Sontum investigated. The MRX115 shell contain 82 mole % of a neutral lipid, 10 mole % of a negatively charged lipid and 8 mole % of a positively charged lipid, giving a net negative charge density of 2 mole % (due to the screening of electrostatic interactions by the PEG component the microbubbles display an overall charge of zero). With this small net amount of charge in the lipid shell MRX115 shows intermediate pressure stability. The Sonazoid® shell, on the other hand, comprises at least 50% negatively charged lipids and displays, by far, the best stability of the three.

Dr. Sontum's conclusion from the studies he personally carried out in 1995 are that, contrary to Mattrey's disclosure, MRX115 and Imagent® are far less suitable as contrast agents because, when used for identification of sentinel lymph nodes, those agents are not stable with regard to pressurization during and after administration. Microbubbles comprising at least 50% negatively charged phospholipids, such as Sonazoid®, in contrast, are unexpectedly and significantly more stable with regard to the same pressurization. Further, unlike positively charged microbbubles, microbubbles comprising at least 50% negatively charged phospholipids, such as Sonazoid®, will not precipitate an immune response when used as contrast agent for the identification of sentinel lymph nodes.

In sum, Mattrey does not disclose, suggest or otherwise contemplate using phospholipid-containing compositions bearing an overall negative charge. Nor does Mattrey appreciate the unexpected stability of such compositions when they are exposed to pressure before, during, and after administration. It would therefore not have been obvious to specifically pick microbubbles comprising at least 50% negatively charged phospholipids to specifically identify sentinel lymph nodes, as presently claimed, even in view of Dugstad. Applicants respectfully submit that the only way that one of skill in the art would pick microbubbles comprising at least 50% negatively charged phospholipids to specifically

identify sentinel lymph nodes, as presently claimed, is by using impermissible hindsight. Since the pending claims are not obvious in view of the combined teachings of Mattrey and Dugstad, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) are respectfully requested.

Applicants conclude, on the basis of the above argumentation, that the pending claims are patentable and requests favorable consideration.

The Examiner is invited to telephone the undersigned in order to resolve any issues that might arise and to promote the efficient examination of the current application.

Respectfully submitted,

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